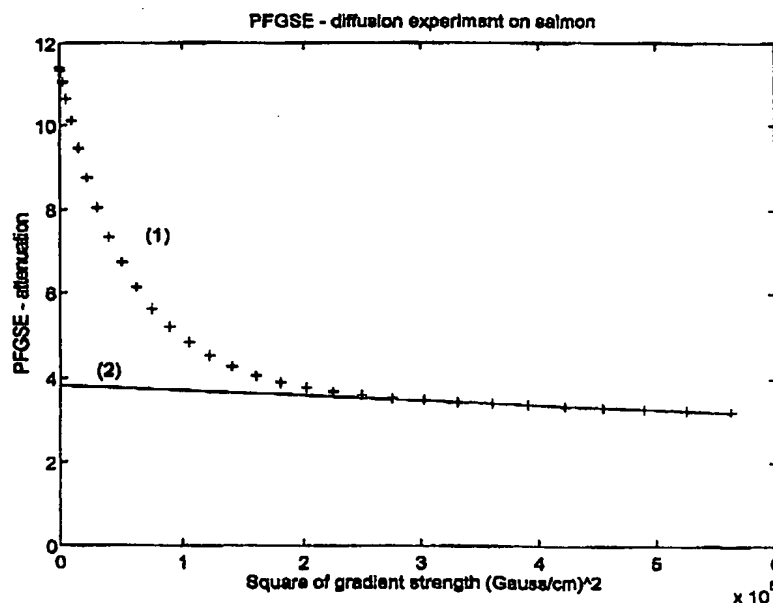


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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/NO99/00082 <b>(22) International Filing Date:</b> 9 March 1999 (09.03.99) <b>(30) Priority Data:</b> 19981517                      3 April 1998 (03.04.98)                      NO <b>(71)(72) Applicant and Inventor:</b> SØRLAND, Geir, H. [NO/NO]; Hagebyveien 32, N-9404 Harstad (NO). <b>(74) Agent:</b> RUDI, Alf-Petter; Jurikon Teknologi AS, Storgata 102, N-9008 Tromsø (NO).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <i>In English translation (filed in Norwegian).</i>

**(54) Title:** A METHOD FOR MEASURING FAT AND WATER CONTENT IN A BIOLOGICAL SAMPLE**(57) Abstract**

The invention is a method for simultaneous determination of the content of fat and water in a biological sample. The method applies nuclear magnetic resonance (NMR) for the determination of fat and water in for example salmon.

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## A method for measuring fat and water content in a biological sample

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The invention is a method for determining the content of water and fat in a biological sample. The method makes use of Nuclear Magnetic Resonance (NMR) to measure the content of fat and water in for example fish or meat.

10

Within the production of articles of food, there is an evident need for a determination of water and fat in the products produced. In the part of the industry which breeds marine animals or fish, salmon in particular, it is important to determine these quantities. The composition in weight of water and fat in the fish, is a factor which indicates the quality of it, and which is decisive for the market. This composition is important for the production of pig as well.

15

There are several methods to measure the fat and/or water content:

One method is the Soxhlet's method where a sample is dried at 105 degree Celsius for 4 hours. The water content is then found by weighing before and after drying. The fat content is found by extracting the fat with a solvent which dissolves the fat from the sample.

20

Another method, Foss-let, homogenises the sample and gypsum is added together with perclorethylene. The gypsum ties up the water while the perclorethylene ties up the fat.

25

A third method uses photometrical techniques and infrared light. This demands an expensive experimental set-up.

30

The present methods involves either an expensive experimental set-up and/or it takes too long to perform the quantification for it to be an alternative within the production of articles of food. Due to this, the breeding industry does not find it necessary to perform the quantification

The purpose of the invention is to provide for a method which fast , with less expensive experimental set-up, and with great accuracy can determine water and fat content in articles of food.

35

This is done by placing the biological sample in a homogeneous/static magnetic field, and expose it to an oscillating magnetic field, which together with a magnetic field gradient over the sample measures the nuclear magnetic moment of the protons, in a diffusion experiment and a combined diffusion/relaxation time experiment. Then the proton signal from fat and water may be separated as they differ in molecular mobility.

40

More details of the invention is given in the following text with reference to the figures.

Figure 1: A biological sample placed in a NMR-spectrometer.

Figure 2: Electromagnetic signals which are imposed using the Pulsed Field Gradient Spin Echo method (PFGSE).

Figure 3: The result from a PFGSE NMR-diffusion experiment on homogenised salmon.

Figure 4: The PFGSE experiment followed by a train of 180 degree RF-pulses, a transversal relaxation time experiment.

Figure 5: Shows the damping of fat signal due to T2\*.

Figure 6: Shows the damping of water signal.

### Theory

- When placing a proton in an external constant magnetic field, the expectation value of the nuclear magnetic moment will be aligned with this field. The Hamiltonian for non-interacting nuclear magnetic spins in an external field can be written

$$\underline{\bar{H}} = \gamma \hbar I H(t) \quad (L1)$$

- where  $\gamma$ =gyromagnetic ratio,  $\hbar$ =Planck's constant.  $I$ =spin-operator and  $H(t)$  is the external magnetic field. The time dependence of  $H(t)$  is included in order to make (L1) valid under influence of the oscillating magnetic field (RF-field) and magnetic field gradients (g). The eigen values or energy levels to the Hamiltonian with a constant magnetic field  $H_0$  is written

$$\underline{E = \pm \frac{1}{2} \gamma \hbar H_0 = \pm \frac{1}{2} \hbar \omega_0} \quad (L2)$$

The gap between the two energy levels is then written

$$\underline{\Delta E = \hbar \omega_0} \quad (L3)$$

At thermal equilibrium one will have a difference in population between upper and lower level given by the Boltzmann-factor

$$\underline{\frac{n_{\text{ovre}}}{n_{\text{nedre}}} = e^{-\frac{\hbar \omega}{kT}}} \quad (L4)$$

- where T is absolute temperature and  $k$ =Boltzmann's constant.  
The implication of a difference in population between the energy levels, is that a system placed in an external magnetic field will have a nuclear magnetic moment which will be proportional to the proton content within the system.

In thermal equilibrium the expectation value of the nuclear moment will be aligned with the external magnetic field. By imposing an oscillating magnetic field (RF-field) with a frequency equal to the resonance frequency between the energy levels degenerated by the constant magnetic field, transitions between the energy levels will be induced (ref.1). The vector of the expectation value will then change from being in thermal equilibrium with the constant external magnetic field to precess in a orbit dependent on the constant external magnetic field and the RF-field.

When switching the RF-field off, the proton system will tend to be aligned with the external constant magnetic field again with a speed given by the characteristic relaxation times T1 and T2. This effect can be recorded by the very same RF-coil which imposed the RF-field. The current induced in this RF-coil will be proportional to the amount of protons. From the RF-coil signal one should therefore be able to quantify the proton content within the system ( see figure 1).

One may also record the mobility of the protons by applying pulsed magnetic field gradients. These gradient pulses with amplitude g, imposes a position dependent frequency on the nuclear magnetic moment which precesses in the plane transverse to Ho.

$$\omega = \gamma H_0 + \gamma g z \quad (L5)$$

By using RF-pulses and magnetic field gradient pulses in a diffusion experiment (ref.2), a dephasing of the net nuclear magnetic moment is given by

$$\varphi = \gamma g (z_2 - z_1) \quad (L6)$$

where (Z2-Z1) is the distance the protons has moved during the experiment. With larger (Z2-Z1), i.e. larger mobility, the induced current in the RF-coil, the NMR-signal, will decrease due to the dephasing. By assuming a gaussian distribution of diffusion coefficients (ref.2), the attenuation of the NMR-signal can be written

$$I = I_0 e^{-\frac{t_1}{T_2}} e^{-\frac{t_2}{T_1}} e^{-\gamma^2 g^2 D \int_0^t \left( \int_0^t g(t'') dt'' \right)^2 dt'} \quad (L7)$$

35

t1 = time NMR-signal is affected by T2-relaxation

t2 = time NMR-signal is affected by T1-relaxation

g(t'') = total magnetic field gradient, applied and internal

D = mean value of diffusion coefficient

40

T1 = characteristic longitudinal relaxation time

T2 = characteristic transversal relaxation time

Io = initial NMR-signal which is proportional to proton content

There are several ways to perform a diffusion experiment by NMR. Here the Pulsed Field Gradient Spin Echo (PFGSE) sequence is used (see figure 2). Corrections for T1 processes is then not necessary, and the NMR-signal will be refocused due to magnetic field inhomogeneities. The echo-attenuation for the PFGSE sequence is written

$$I = I_0 e^{-\frac{2\tau}{T_2} - \frac{2\tau^3}{3} \gamma^2 G_i^2 D} e^{-\gamma^2 g^2 D \delta^2 (\tau - \frac{\delta}{3})} \quad (L8)$$

where  $G_i$  is the internal magnetic field gradient caused by susceptibility changes over the heterogeneous sample,  $g$  is the applied magnetic field gradient,  $\delta$  is the gradient pulse length, and  $\tau$  is the time interval between the 90-degree RF-pulse and the 180-degree RF-pulse.

The two first terms in first exponent of (L8) are collected to form a  $T2^*$ -term

$$I_{(T2^*)} = I_0 e^{-\frac{2\tau}{T_2} - \frac{2\tau^3}{3} \gamma^2 G_i^2 D} \quad (L9)$$

The echo attenuation is then written

$$I = I_{(T2^*)} e^{-\gamma^2 g^2 D \delta^2 (\tau - \frac{\delta}{3})} \quad (L10)$$

The relaxation term and diffusion term due to internal magnetic field gradients are thus collected in  $I(T2^*)$ .

To separate between water- and fat signal one makes use of the fact that water and fat differ significantly in molecular mobility. With a PFGSE experiment one will then be able to separate the two signals (see figure 3). By fitting the two signals to (L10) with different  $D$ , one will have values of  $I(T2^*)$  for fat and water separately.

After having separated the signals, one must correct for  $T2^*$ -effects. This is important as a PFGSE-sequence may last for 10 milliseconds, and the  $T2$ -relaxation time for fat and water are of the same order of magnitude. A successful quantification is therefore dependent on a proper  $T2^*$ -correction as to insure that all NMR-signal from fat and water signal is included.

## Corrections for T2- and G<sub>i</sub>-effects (T2\*)

5 NMR-signal which is lost during the PFGSE-sequence can be corrected for by measuring T2\* and make an extrapolation to zero observation time (see figures 5 and 6). This is done by a combination of the PFGSE-sequence succeeded by a train of 180-degree RF-pulses, a T2\*-relaxation experiment (see figure 4). This experiment is performed for two values of the applied magnetic field gradient g:

10 a) g so strong that water signal is suppressed. One may then measure T2\* for fat and I<sub>0</sub>=I<sub>fat</sub>, and a quantification of fat is finished.

15 b) g so small that water and fat is measured simultaneously. τ<sub>c</sub> can then be set to be less than τ such that one has a measuring point close to zero observation time. τ<sub>c</sub> cannot be set to be 0 as proton NMR-signal from the protein in the system will contribute. Proton in protein has a T2 of 10-100 μs (ref.3) such that the smallest τ<sub>c</sub> which can be used is around 250 μs. The first measuring point will then be at a observation time of 0.5 ms, and the proton NMR-signal from the protein will then have relaxed to an insignificant amount. As one knows T2\* for fat and I<sub>fat</sub> from a), the T2\* and I<sub>0</sub>=I<sub>water</sub> for water can be found. Quantification of water is then finished

20 In case a) the attenuation is written

$$\underline{I = I_{fat} e^{-\left(\frac{1}{T_2} - \frac{\tau^2 \gamma^2 G_i^2 D}{3}\right) 2(n+1)\tau}} \quad (L11)$$

25 A weighted linear fit of the logarithm of (L11) to the function

$$\underline{y = -ax - c} \quad (L12)$$

will then result in a value

$$30 \quad \underline{I_{fat} = e^c} \quad (L13)$$

35 With a weighted fit one takes into consideration that (L12) is not valid at all times. When the observation time approaches zero, (L12) is at its highest validity. The first measuring points are therefore given more weight than the latter ones. Figure 5 shows the attenuation of the fat-signal due to T2\*-relaxation processes.

40 Diffusion- and relaxation-effects in the NMR-signal arising from protons in fat is now corrected for, and the resulting signal is a measure for the content of fat within the system.

In order to perform a similar fit for the T2\*-experiment where signal from water and fat is present simultaneously, one must first subtract the signal arising from the fat. This is done by scaling the attenuation from the fat signal in a) with I(T2\*). I(T2\*) is the extrapolated value at 0 applied magnetic field gradient. The extrapolation to I(T2\*) is visualised with a solid line in figure 3. Effects from diffusion of fat during the PFGSE-sequence is then corrected for, and the resulting T2\*-attenuation is from water alone.

A weighted linear fit to the function in (L12) then yields

$$\underline{I_{vann} = e^c} \quad (L14)$$

The system is weighed prior to the NMR-experiments. When one has found the weight content of fat and water, the weight of the rest will consist of protein and carbon.

## Documentation

The method is tested on homogenised salmon where the experimental results are displayed in figures 3, 5 and 6. Control measurements have been performed using the Foss-let method.

The result from the two different methods result in approximately the same water content while a higher fat-content was found using the NMR-method.

## References

Ref.1: *NMR-Signal Reception: Virtual Photons and Coherent Spontaneous Emission*, Concepts Magnetic Resonance 9: 277-297 (1997).

Ref.2: *Pulsed-Field Gradient Nuclear Magnetic Resonance as a Tool for Studying Translational Diffusion: Part 1. Basic Theory*, Concepts Magnetic Resonance 9: 299-336 (1997).

Ref. 3: *A review of H nuclear magnetic resonance relaxation in pathology: Are T1 and T2 diagnostics?*, Medical Physics 14 (1), Jan/Feb 1987.



## Claims

- 5 1. A way to measure the water and fat content in a biological sample, characterised by a sample placed in a homogenous/static magnetic field and affected by an oscillating magnetic field (figure 1), which together with a magnetic field gradient measures the nuclear magnetic moment of the protons, in a combined diffusion experiment (figure 2) and a combined diffusion/relaxation experiment (figure 4), as one may resolve the fat signal from the water signal due to their difference in molecular mobility.
- 10 2. A way to measure the water and fat content in a biological sample according to 1., characterised a controlled separation of the fat signal and the water signal by changing the magnetic field gradient to produce a signal with water and fat signal simultaneously present and fat signal present only (figure 3), as the water signal is found by subtracting the two sets of signal.
- 15 3. A way to measure the water and fat content in a biological sample according to 2., characterised by a T2\*-relaxation time correction of the signal from fat and water separately by doing the following:
  - 20 i) The magnetic field gradient so strong that fat signal is present only, and a T2\* correction is performed on fat signal only (figure 5, L13).
  - 25 ii) The magnetic field gradient so weak that a T2\*-relaxation time curve is recorded for fat and water signal present simultaneously
  - 30 iii) A scaled version of the T2\*-relaxation time curve for fat only which is corrected for diffusion effects in the diffusion experiment is subtracted from the T2\*-relaxation time curve recorded for fat and water in ii), and one is then left with the T2\*-relaxation time curve for water only (figure 6, L14).
- 35 4. A way to measure the water and fat content in a biological sample according to 1,2 and 3 , characterised by the use of magnetic field gradients in order to generate a Fourier-Transformed NMR signal which results in one dimensional resolution of the biological sample, such that a reference sample can be measured simultaneously with the unknown sample.

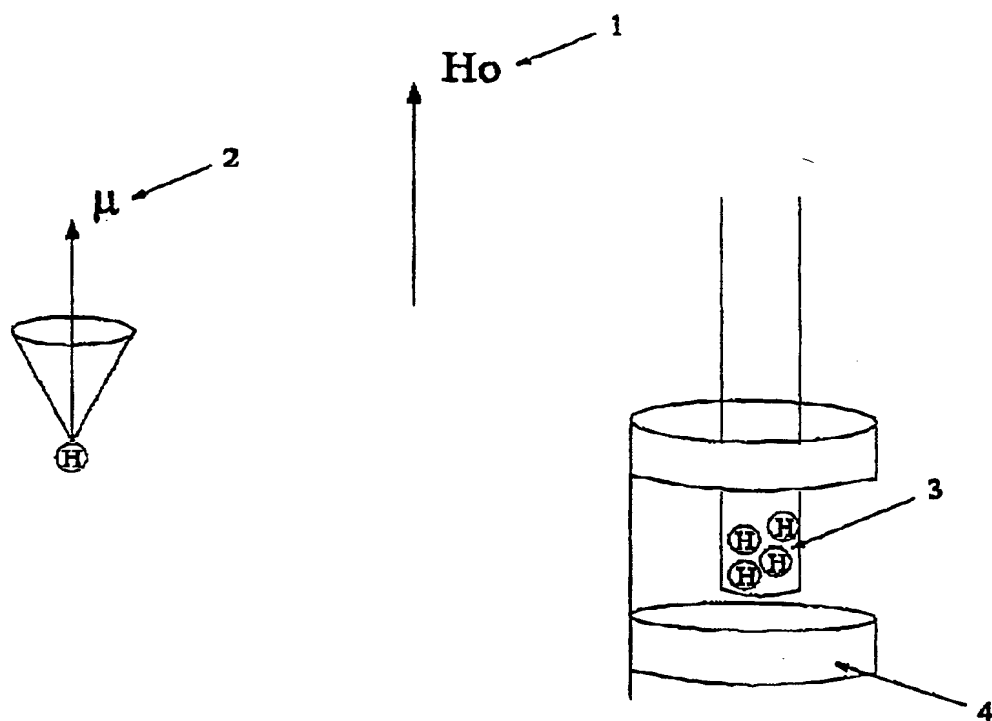


Figure 1

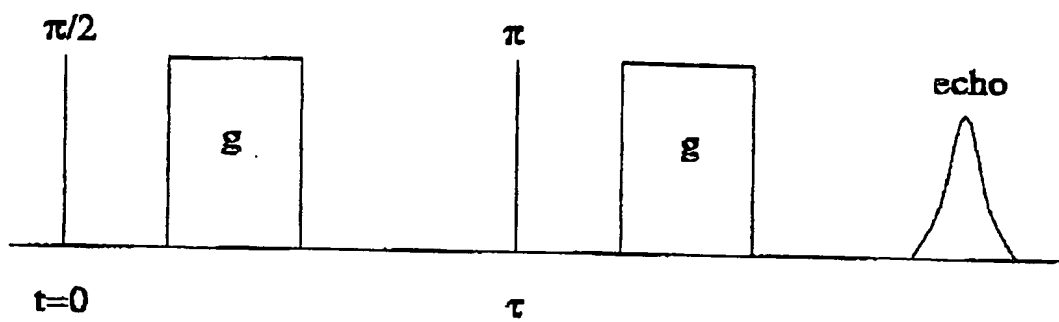


Figure 2

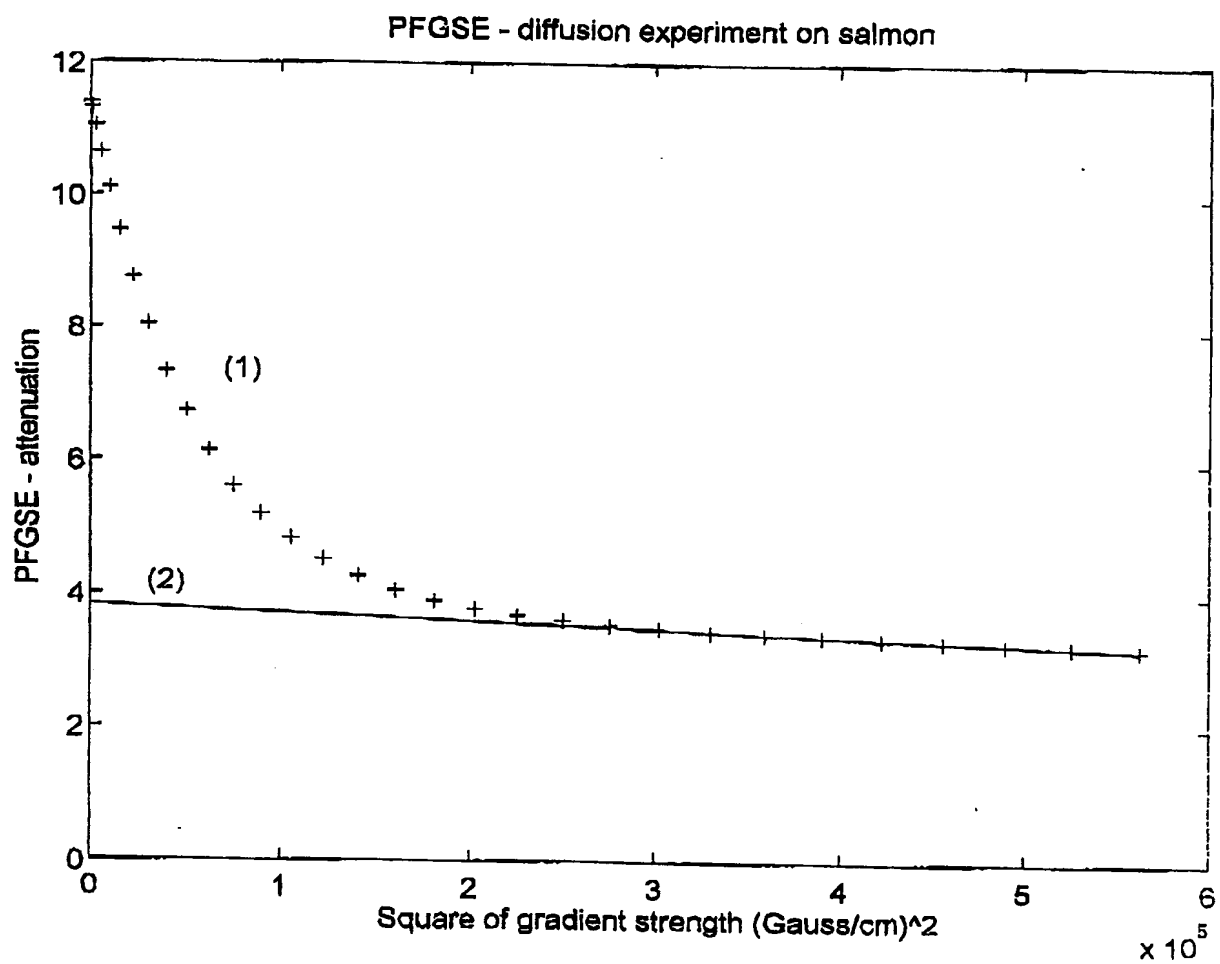


Figure 3

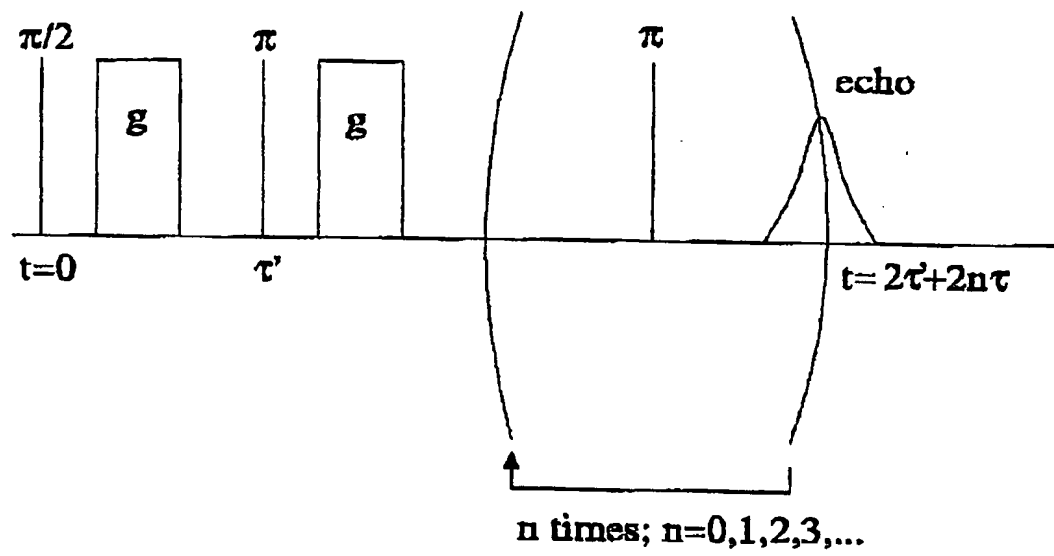


Figure 4

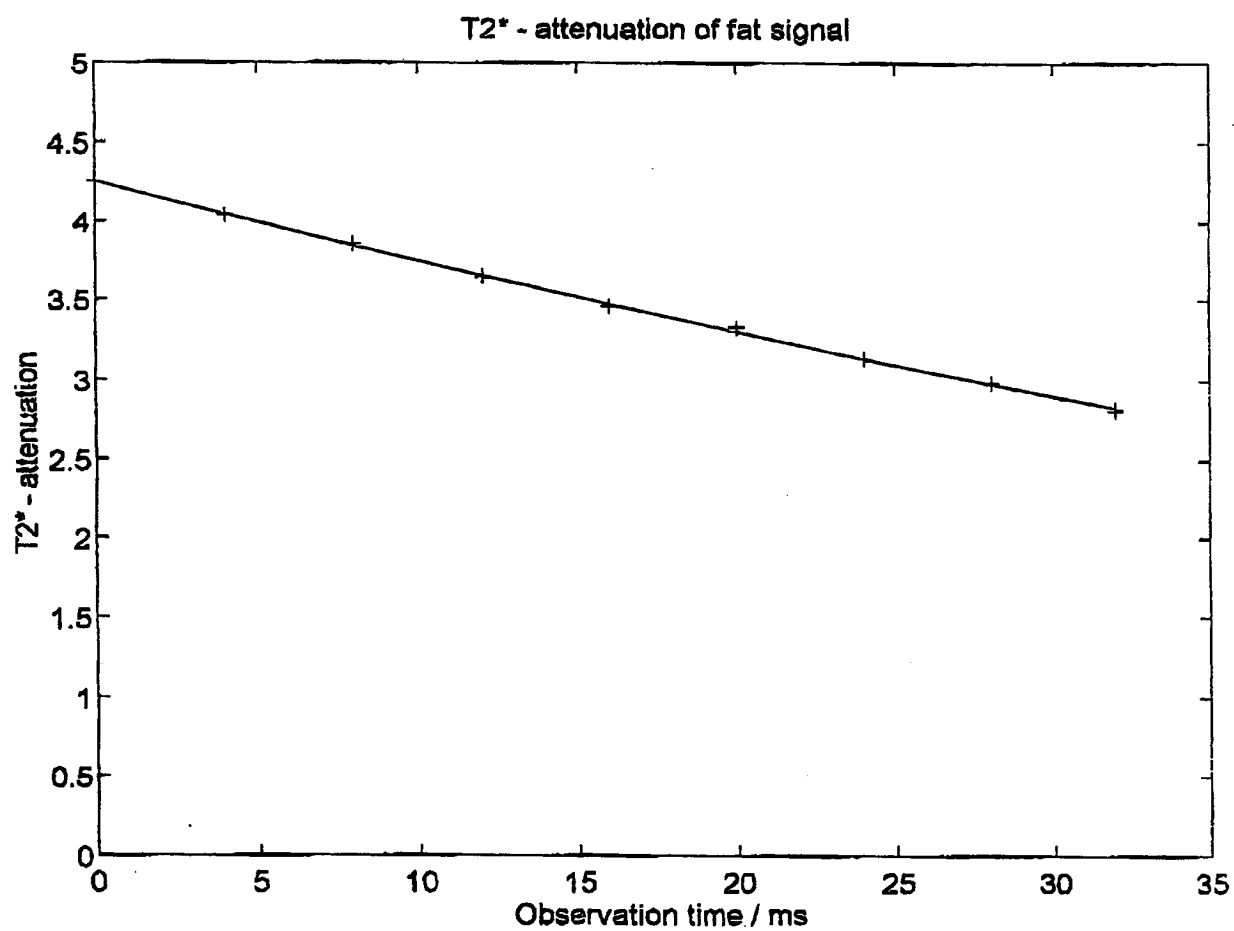


Figure 5

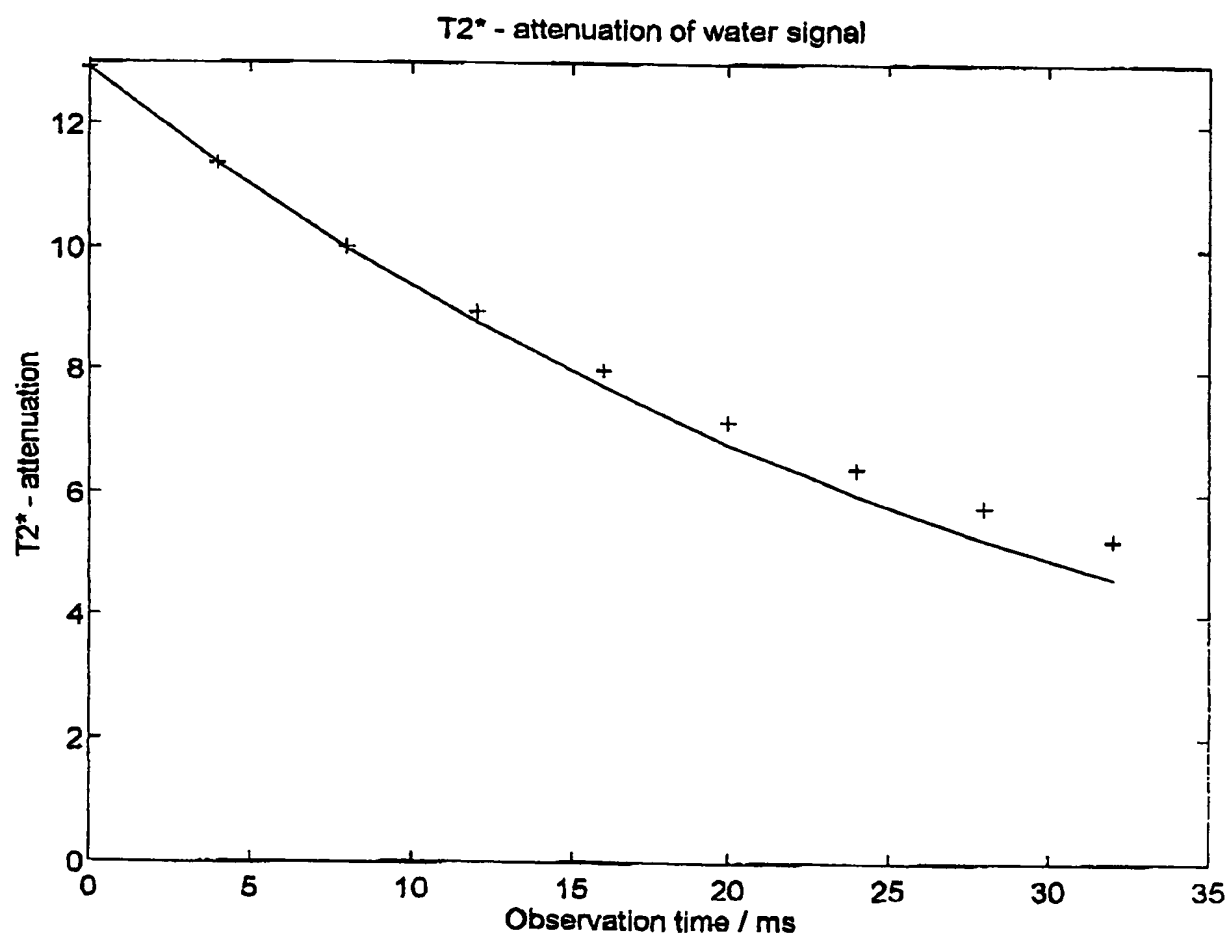


Figure 6

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 99/00082

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01R 33/54, G01R 33/483

According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SU1043537 A (LENGD REFRIG IND) 1983-09-23 (abstract) World Patents Index (online). London, U.K.: Derwent Publications, Ltd. (retrieved on 1999-08-19). Retrieved from: EPO WPI Database. DW8425, Accession No. 84-156454	1,2,4
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X	JP1190342 (TOSHIBA CORP) 1989-10-18 (abstract). (online)(retrieved on 1999-08-19). Retrieved From: EPO PAJ Database	1,2,4
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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5134372 A (Y. INOUE), 28 July 1992 (28.07.92), abstract	1,2,4
A	---	3
A	US 5322682 A (G. BARTZOKIS ET AL.), 21 June 1994 (21.06.94), abstract	3
A	GB 2261072 A (BRUKER ANALYTISCHE MESSTECHNIK GMBH), 5 May 1993 (05.05.93), abstract	1-4
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**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

02/08/99

International application No.  
**PCT/NO 99/00082**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5134372 A	28/07/92	DE 68927053 D,T EP 0417284 A,B JP 1303137 A JP 1707855 C JP 3070965 B WO 8911822 A	30/01/97 20/03/91 07/12/89 27/10/92 11/11/91 14/12/89
US 5322682 A	21/06/94	WO 9403109 A	17/02/94
GB 2261072 A	05/05/93	DE 4133643 C	03/12/92